APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1976, p. 327-331
Vol. 31, No. 3

Printed in U.S.A.

New Type of Turbidostat with Intermittent Determination of Cell Density Outside the Culture Vessel

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Received for publication 15 July 1975

An inexpensive turbidostat featuring intermittent determination of optical density in an interchangeable measuring tube and incorporating electronic circuits for the automated mechanics is described.

Although continuous culturing in flow-through systems is the only reliable method in which "...the mean physiological state of the population can be stabilized by maintaining a constant rate of cell increase in constant conditions" (3), batch culturing in static systems still appears to be the most commonly used technique in algal research. The simple reason for this apparently contradictory phenomenon is the high cost of most of the commercially available automatic equipment, which restricts its use to highly specialized fundamental physiological and biochemical research.

Two types of automatization have been developed. In the turbidostat the flow of fresh medium is regulated by a photocell sensor to keep a constant cell density in the culture vessel (8, 9, 14). In the chemostat the cell density is controlled by constant dilution of the contents of the culture vessel by a nutrient-limiting medium (5, 7, 12).

The turbidostat, on the contrary, will only dilute the culture when a preset optical density is exceeded. One of the major problems with turbidostats is, however, the fouling of the vessel walls (8), which often results in an uncontrolled decrease of the optical density of the culture.

Various ingenious devices and techniques aiming to solve this particular problem have been described: e.g., mechanical wipers and scrapers (1, 2, 10, 11), coating of the glass surfaces (6), and intermittent turbidimetry with vigorous aeration and stirring during the non-checking periods (4, 6). According to Ricica (15) none of these techniques can, however, completely prevent or eliminate the fouling. Consequently the entire culture has to be transferred periodically to a clean vessel. Measuring the algal density in an external tube (outside the culture vessel) partly solves the problem, since the latter tube can easily be cleaned or replaced without disturbing the culture itself. Evans et al. (5) and Shelef et al. (16) continuously circulate the culture suspension through an external flow-through tube, which can, at appropriate moments, be separated from the culture vessel and flushed out with NaOH and distilled water.

Besides these technical problems, the available turbidostats are quite expensive, due to the complexity of the measuring equipment and to the use of stirrers, peristaltic pumps, magnetic valves, etc.

Keeping the existing systems with their respective problems in mind, and trying to reduce their technological complexity as much as possible, we were able to design a simple and inexpensive turbidostat. There are two major innovations in our system as compared with the existing equipment: (i) an intermittent measuring of the algal density in a lateral (easily interchangeable) tube, which is filled just prior to the measurement and emptied immediately afterwards to prevent fouling; and (ii) the use of compressed air instead of mechanical systems to "execute" all the operations as commanded by the electronic control.

MATERIALS AND METHODS

The turbidostat shown in Fig. 1 consists of four distinct parts: three glass serum bottles, i.e., a culture vessel, A (13), a somewhat larger stocking vessel, B, for the culture medium, and a small dispenser vessel, C; and the electronic control unit, D. All three bottles are inverted on their iron clamps and their volumes can be selected at will. For our research purposes, we used bottles of 1, 2, and 0.2 liters, respectively, for vessels A, B, and C.

The algal vessel, A, is provided with a lateral communicating tube, 1 (6 mm in diameter), a lateral overflow tube, 2, and an inlet, 3, for inflow of the culture medium. A drain tube with stop-cock, 4, an air inlet tube, 5, and an air outlet tube, 6, are fitted into the rubber stopper. Compressed air eventually

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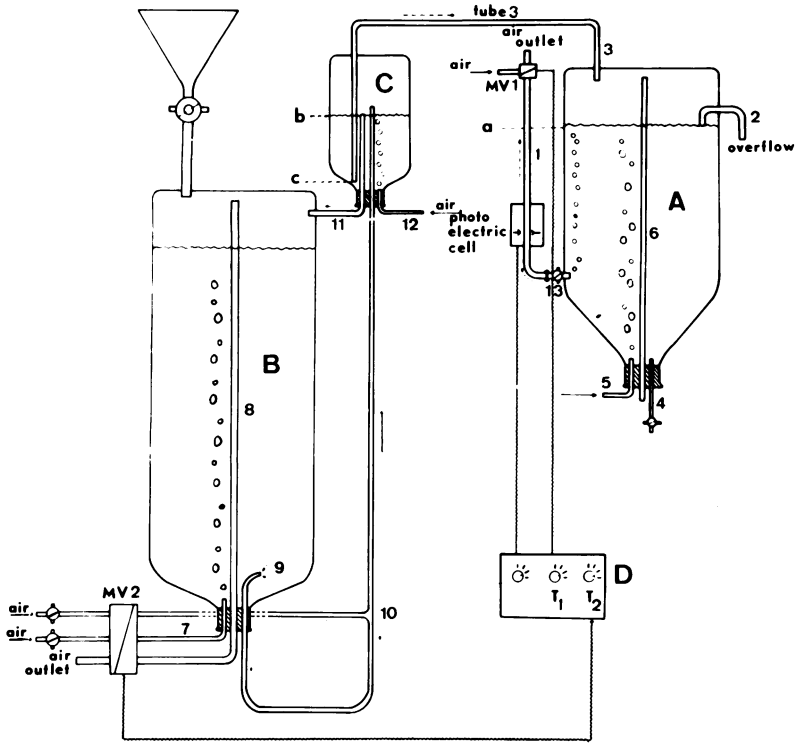

FIG. 1. *Schematic diagram of the turbidostat: (A) algal culture vessel; (B) stock medium; (C) dispensor vessel; (D) electronic control unit.*

enriched with CO_2 bubbles continuously through the culture, keeping the algae in suspension and at the same time providing the necessary carbon dioxide for photosynthesis.

A photoelectric cell in a small box is fitted around the lateral tube, which is connected at its end to a 2/3 electro-magnetic valve, MV1. Normally the valve is open in the "air inflow" position, meaning that compressed air flows through the tube into the algal vessel, producing gentle bubbling. When magnetic valve MV1 is energized through the electronic control unit (D), the valve switches to the air outlet position, cutting off the air inflow and at the same time allowing the algal suspension to rise from the bottle into the lateral connecting tube up to the level of the suspension inside.

Stocking vessel B, which can be filled from the top through a glass funnel, has a rubber stopper through which three tubes enter the flask: (i) an air inlet tube, 7, for bubbling of air to keep all solutes homogeneously distributed; (ii) an air outlet tube, 8; and (iii) a draining tube, 9, by which the medium is continuously raised by an air-water lift, 10, to vessel C.

The air inlet and outlet tubes, as well as the air tube to air-water lift 10, are connected to an electromagnetic valve, MV2 (with three gangways). When not energized, the gangways are open, allowing air to bubble to the stocking vessel, with outflow through the air outlet, and air-water to rise to vessel C.

Dispenser vessel C is provided with three tubes through its stopper and another one, tube 3, that passes through the top wall to algal culture vessel A. Through air inlet tube 12 air bubbles through the medium as in vessel B. (We should like to mention that air bubbling can be omitted when using very pure solutions. In this case, the air inlet tube into bottle B is left out; in vessel C, however, tube 12 has then to be extended above water level b.) Air-water lift tube 10 raises the medium from vessel B to vessel C, and overflow tube 11 drains the culture medium exceeding level b back into vessel B. When MV2 is energized, air-water lift 10 and all air inflow and outflow in B are cut off. The continuous air bubbling in dispenser bottle C creates an overpressure and forces the culture medium between levels b and c to flow into algal vessel A, with overflow of the same volume of algal suspension via siphon 2.

Electronics (see schematics in Fig. 2). Every preset time, e.g., each 30 min, an electric clock commands the densitometric operation. When energized, timer T_1 closes magnetic valve MV1 during time lapse t_1 (adjustable between 2 and 20 s by knob T_1) and operates the fet switch, which delays the measuring of the optical density for 5 s (this period is sufficient to allow a standstill of the culture suspension in the lateral tube).

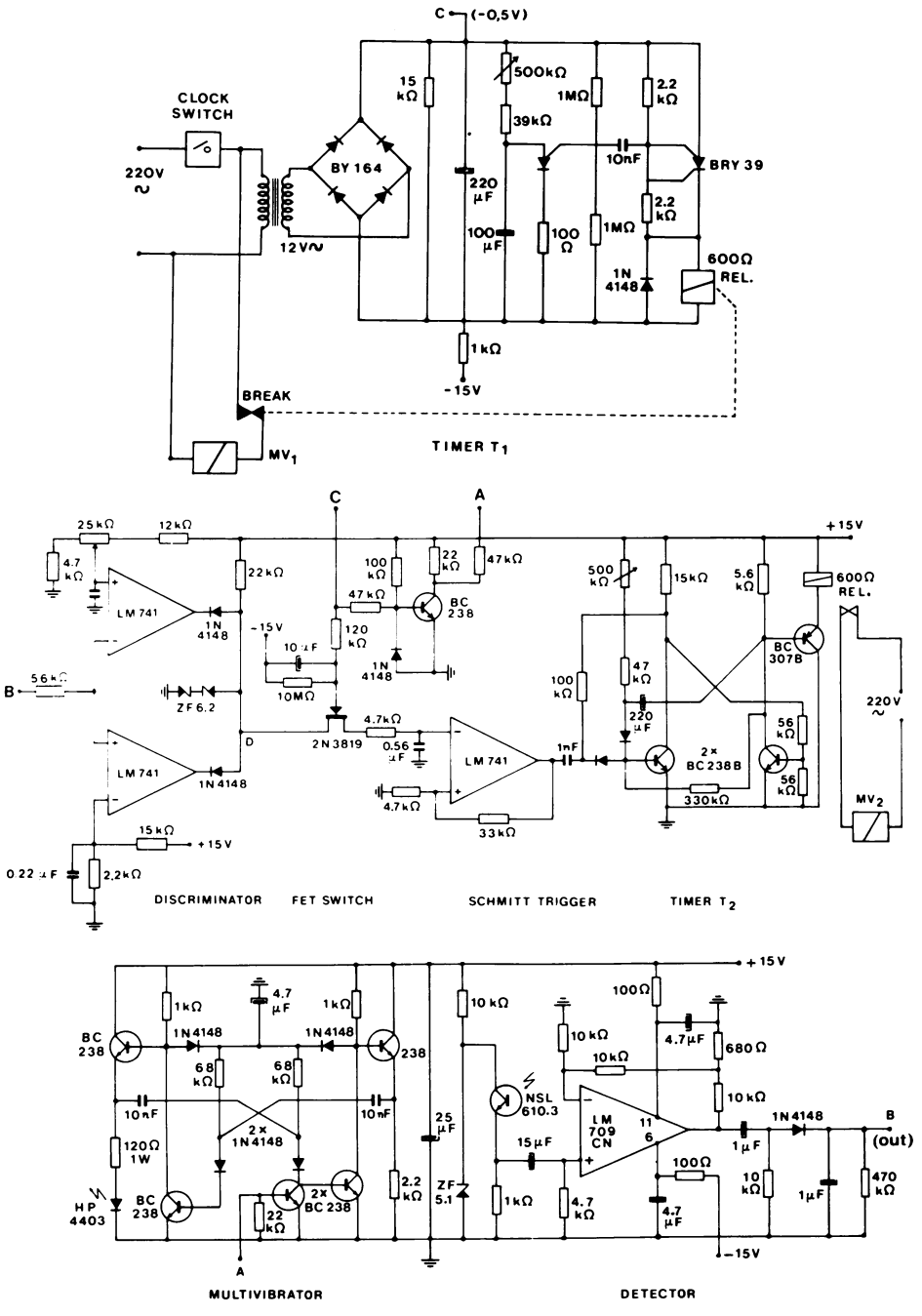

FIG. 2. *Circuit diagram of the electronics of the turbidostat.*

When T_1 is energized, the voltage at C (V_C) increases from -15 to -0.5 V, which lowers voltage V_A sufficiently to trigger the multivibrator; as a consequence the light emission diode radiates pulsed light onto the phototransistor of the discriminator.

The light transmitted through the culture suspension in the lateral tube generates an AC signal, which is amplified and rectified, and gives voltage V_B. Five seconds after the start of the operation, voltage V_B is compared with the voltage range preset on the discriminator.

The upper voltage limit of the latter is fixed,

whereas the lower one is variable. The upper limit corresponds to maximal light transmission (i.e., the minimal algal concentration), and the lower limit corresponds to minimal light transmission, at which dilution of the culture can proceed.

When V_B is situated between these limits the discriminator output, V_D, is positive and timer T_2 will operate magnetic valve MV2 during a period, t_2, adjustable between 5 s and 2 min by knob T_2, leading to a dilution of the culture medium. In the meantime, T_1 has switched MV1 off and lateral tube 1 is emptied; consequently V_B decreases to about 2 V, V_D becomes negative, and the Schmitt trigger is reset (output + 15 V). When the clock switch is finally reopened, all other voltages drop to their initial values.

RESULTS AND DISCUSSION

Theoretical considerations. Due to the automatic flushing after each measurement, the lateral tube of the culture vessel is filled with algal suspension for only a few seconds during preset period T_1; since the latter tube is coated with a silicone agent, fouling is reduced to an absolute minimum.

The tube can easily be replaced by closing stop contact 13 and disconnecting the tube, which is fitted to the magnetic valve and to stop-cock 13 by plastic tubing.

For axenic culturing, the different items of the apparatus can be sterilized and the compressed air can be filtered. To keep bacterial development as low as possible, bottles B and C are hung in a refrigerator. It is quite clear that fluctuations of the cell density in the culture bottle are dependent on the constancy of the culturing parameters, such as light, temperature, and volume of CO_2 (thus, the volume of air bubbled through the algal suspension).

The amplitude between the minimal and maximal cell concentrations (respectively, immediately after and just prior to dilution) is a function of several variables: (i) the volume of the inflowing medium from the dispenser bottle; (ii) the interval between two successive measurements; and (iii) the normal culturing parameters: light, temperature, CO_2, nutrient concentration of the stock medium, etc.

The interval, i.e., the time lapse between successive measurements, determines the culture growth in excess of the preset density (as selected by sensitivity knob S). Indeed, if a measurement takes place at t_1 at a cell density, X_1, slightly inferior to the preselected density X_s ($X_s - X_1$ = extremely small), there will be no dilution.

Until the next measuring, t_2, the culture will, however, grow to a density, X_2, that will exceed density X_s. The excess $X_2 - X_s$ is a function of the interval between t_2 and t_1 and is, of course, strongly dependent on the previously mentioned culture conditions.

Practical applications. For 1 year, algological research has been carried out to test the ability of the new turbidostat to keep the cell density within a narrow range at different concentrations.

In the example given below the marine Prasinophycea *Tetraselmis suecica* was kept in the exponential growth phase at a concentration close to 10^6 cells/ml. The culture medium was artificial seawater of 70‰ salinity enriched with a 1% nutrient solution of Vlasblom (13). The culture bottle was kept in a temperature-conditioned room at 25 ± 1 C and illuminated at approximately 2,000 lux by two fluorescent tubes.

To check the cell concentration samples of 5 ml were taken at random intervals during a 1-week period.

The cell density of the samples and the volume of fresh medium pumped into the algal vessel between two successive random samplings are given in Fig. 3. From this graph it is clear that the cell density fluctuated around the original preset value within a narrow range (0.8×10^6 to 1.2×10^6 cells/ml).

In total the dilution was 2,100 ml; the volumes of added medium corresponded quite well with the theoretical dilutions, being 300 ml/day.

An analogous experiment with another marine phytoflagellate, *Dunaliella viridis*, revealed a fluctuation of the cell density between 2.8×10^6 and 3.2×10^6 cells/ml. Furthermore, the equipment proved suitable for fundamental studies on the uptake of nitrogen and phosphorus by the freshwater coenobiar Chlorophyceae *Scenedesmus acutus* from nutrient solutions

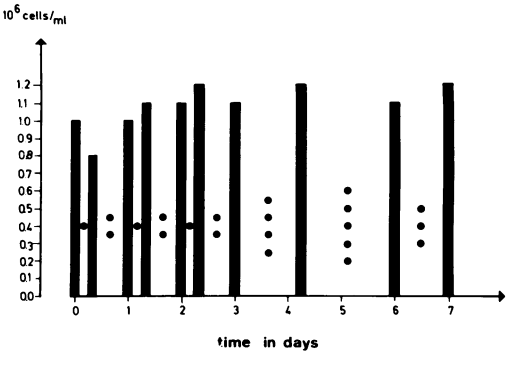

FIG. 3. *Algal density of random samples and total dilution volume between two successive samplings (●, one 100-ml dilution).*

(A. Windels, Lic. thesis, State Univ. of Ghent, Ghent, Belgium, 1975).

For this purpose we have fitted a third magnetic valve, MV3 to overflow tube 2 and connected the other end to a fraction collector by plastic tubing. When the photoelectric cell commands dilution to the control box, the operational sequence is as follows. Interval T_2 (originally preset at 2 min is split into three subperiods: (i) the sample collector is commanded to move forward one sample position; (ii) magnetic valve MV3 opens for about 1 min, draining the algal suspension exceeding level b to the fraction collector; and (iii) as in the normal operations, magnetic valve MV2 closes for 1 min to siphon 100 ml of culture medium into the algal vessel, thus diluting the culture about 10%.

To prevent algal as well as bacterial growth, with subsequent changes in the chemical composition of the effluent, the algal suspension flowing into the fraction collector is cooled at 0 C. As the intervals between the successive dilutions are registered on a potentiometric recorder and the cell concentration in the samples is determined, the growth rates of the algae can be determined.

ACKNOWLEDGMENTS

P. Sorgeloos is an "aspirant" at the Belgian National Foundation for Scientific Research (N.F.W.O.).

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